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EXAMINER	
SODERQUIST, ARLEN	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/048,244	BLUMENTHAL, DONALD K.	
	Examiner	Art Unit	
	Arlen Soderquist	1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 April 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7,10 and 14-35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7,10 and 14-35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

1. Examiner notes the following. Page 8, lines 6-14 of the instant specification equate the acronym "KID" to be a peptide based on the "Kinase Inducible Domain" of an enzyme substrate. This is the portion of the substrate that undergoes the chemical modification by the enzyme. Thus for examination purposes the acronym "KID" is being treated as any Kinase Inducible Domain.

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 1-7, 10 and 14-35 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant specification teaches a single dilabeled molecule based on sequence ID No. 5 for which data was shown confirming the change in fluorescence upon covalent modification of the molecule. This clearly shows that applicant was in possession of a means for assaying for at least one enzyme. However, the only means for assaying for a substrate that is taught in the instant specification requires a plurality of different dilabeled molecules that are brought into contact with the enzyme to determine or identify if the enzyme will cause a change in the fluorescence upon covalent modification of one or more of the plurality. Thus, applicant was not in possession of a means for assaying for a substrate using a single dilabeled molecule. Another way if interpreting the current means language is that the means for assaying a substrate is covalently by the enzyme substrate. There is no disclosure that would point to applicant being in possession of means for assaying an enzyme substrate by covalent modification of the dilabeled molecule by the substrate. Thus applicant was not in possession of means for assaying a substrate ad this language in the independent claims constitutes new matter. This is a rejection based on the written description. For examination purposes, this language will be treated by examiner as the core molecular backbone includes any sequence having a covalently modifiable portion, because a library of compounds does not appear to be limited in its structure to include any specific structure. If applicant wishes to limit

the structure, specific limitations either regarding the sequences included or the properties need to be included as positive recitations in the claims. The following claim is an example of what the examiner means as far as specific limitations. Although it may still suffer from the enablement problems outlined below or be anticipated by or obvious in view of the art, this example does not suffer from the new matter problems outlined above.

A biomolecular substrate comprising:

a core molecular backbone including a covalently modifiable section, wherein covalent modification of said section without cleaving the core molecular backbone converts the core molecular backbone from a first conformation to a second conformation;

a first dye covalently attached to the core molecular backbone, said first dye is a fluorescent dye; and

a second dye covalently attached to the core molecular backbone with the covalently modifiable section of the core molecular backbone between first and second dyes, wherein when the core molecular backbone is in the first conformation the first and second dyes form a dissociable, ground state-quenched intramolecular dimer and when the core molecular backbone is in the second conformation the first and second dyes physically dissociate resulting in dequenching of fluorescence from at least the first dye.

4. Claims 1-7, 10, 14-33 and 35 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the specific compound(s) disclosed, does not reasonably provide enablement for a scope covering all doubly labeled compounds that have at least a part of their fluorescence quenched through ground state interactions. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. To meet the claim limitations, it requires at least four things to be present: a molecular backbone, a group or position in the backbone capable of being covalently modified that causes a change in the conformation of the molecule, a fluorescent dye and a second dye that are both covalently bonded to the molecular backbone in a configuration that the second dye quenches the fluorescence of the fluorescent dye through a ground state interaction before covalent modification of the molecule and allows fluorescence after covalent modification of the molecule. In the examples shown the molecule includes six parts to produce the working

example, a sequence that includes the chemical modification site (the phosphorylation site), an amino acid replacement on the N-terminus end, an extension of the sequence at the C-terminus, an additional extension of the sequence at the N-terminus and the two dyes covalently attached at the resulting C- and N-terminus locations. Applicant has not shown is whether another structure having more or fewer amino acids (sequence ID No. 7) in the sequence (sequence ID No. 5) will work. Also not shown is if the added labels can be added at any other location(s) along sequence ID No. 5 and still provide a molecule that quenches through ground state physical interactions between the two dyes and has a different conformation that removed the quenching after chemical modification. Finally, the specification fails to show that working compounds can be made for other specifically disclosed sequences – sequence ID Nos. 1, 2, 3, 4 and 9. The specification alleges that other similar sequences can be made for other enzymes, however no actual examples were given. Thus, the instant specification has failed to provide data showing that other sequences can be made that will have partial quenching through ground state-quenching (a non-fluorescence resonance energy transfer mechanism) prior to chemical modification and dequenching after chemical modification. Since the claims only require that a portion of the fluorescence be quenched through the ground state physical associations, other mechanisms may also be present and evidence should be provided to show that the ground state quenching mechanism is at least partly responsible for the quenching. The cited Lakos and Sillen references teach that fluorescence lifetime data is the accepted method of determining the amount of fluorescence quenching due to the various processes. As evidence of the predictability problems that this causes, applicant is directed to the cited Schobel reference. In this reference a donor-acceptor dye pair (Cy5 and Cy5.5) are taught. This dye pair is a resonance energy transfer (RET) dye pair having almost perfect overlap of the Cy5 emission and the Cy5.5 absorption spectrum. However, in at least one embodiment of the pair about only 50% of the quenching results from resonance energy transfer and the remaining quenching is due to static (ground state) quenching processes. From this it either appears that the presence of ground state quenching mechanisms is either quite prevalent in the resonance energy transfer dye pairs or cannot be determined or designed with any high level of repeatability or expectation. Next applicant is directed to the newly cited Mizukami reference. In this reference, figure 1 shows a synthetic fluorometric molecule having two dyes separated by a core molecular backbone.

Figure 2 shows that the fluorescence of the molecules is nearly quenched before contact with the enzyme (see the open circles). The paragraph bridging the columns of page 359 teaches that the fluorescent molecules were initially designed as molecules subject to fluorescence resonance energy transfer quenching in which the fluorescence of the donor is quenched and the fluorescence of the acceptor is observed. When the compounds were excited in aqueous solutions little or no fluorescence was observed from either fluorophore. However in another solvent fluorescence resonance energy transfer was observed. Thus in this case fluorescence resonance energy transfer was expected, but was not seen because of hydrophobic intramolecular interactions (non-fluorescence resonance energy transfer quenching) occurred between the two labels covalently attached to the core molecular backbone. In other words the references show that there is either a high level of expectation of the presence of ground state quenching interactions occurring or there is a low predictability of when these interactions will be present in any given dye pair/molecular configuration.

An additional difficulty with the claimed molecules is that the molecule must have the ability to be covalently modified leading to a configuration change that will cause a change in the ground state quenching mechanisms. All of the dye combinations found in the dependent claims can be used in situations in which there is allegedly FRET involved as evidenced by the instant and past arguments that applicant has provided against the applied references. Each of the anticipatory references and at least one of the references applied to show the obviousness of the claims has a molecular backbone with a site that can be modified to change the conformation. In at least the anticipatory references, there is a clear change in the fluorescence quenching when this occurs. In spite of this applicant argues that the mechanism is either different or identified by the reference as different from the claimed quenching mechanism. The above described Mizukami reference at least partially shows that non-fluorescence resonance energy transfer quenching can occur in situation in which fluorescence resonance energy transfer quenching is expected. In addition to this examiner directs applicant to the newly cited Nishikata reference. In this reference a molecule is taught having two dyes (one fluorescent and one non-fluorescent) separated by a core molecular backbone. The core molecular backbone has a tyrosine with a phosphate covalently bonded thereto. In the experimental section on page 386 under the synthesis of substrates heading it is clear that the disclosed peptide is similar to a peptide used in

a kinase assay and can be prepared through the phosphorylation of a peptide by a kinase. Thus the changes in fluorescence when the molecule is dephosphated are relevant to the changes that would be expected during phosphorylation by a kinase. Scheme 1 and the abstract clearly teach that there is little change in the fluorescence upon dephosphorylation. This shows that there is little change in the conformation of the peptide as a result of either phosphorylation or dephosphorylation. In other words a dilabeled molecule having a covalently modifiable section in a core molecular backbone sequence, does not lead to an expectation that there will be an observable change in the molecule conformation upon covalent modification of the core molecular backbone. Thus designing a compound that is at least partly non-FRET quenched is not an easy task even if one uses core molecular backbones known to have structures with a covalently modifiable sequence or similar in structure to a known covalently modifiable sequence. The above examples show that the predictability of producing a molecule with all of the required elements for the intended use is low. Thus there is an undue level of experimentation required to find other workable embodiments. Additionally or in support of this, applicant has not shown that their own work has produced compounds beyond those that are exemplified in the instant specification or compounds developed by others either on their own or as a benefit of applicant's teachings, that are within the instant claim scope and not exemplified in the instant specification. Thus the instant disclosure in not enabled beyond the specific examples shown in the instant specification. Because of this problem and the fact that the claims require that the quenching be only at least partly due to ground state quenching/association, the examination will treat a reference teaching all but the presence of ground state quenching mechanisms as inherently anticipatory of the claims that it otherwise discloses. Particularly if the dye combination is within the scope claimed and the molecular backbone has a modifiable site therein the compound will be treated as having met the "at least partly" language of the claims in view of the discussion above relative to the cited Lakos and Sillen references.

Relative to claim 27 there are no examples showing compounds including nucleic acids in the core molecular backbone.

5. Claims 14, 25, 27 and 34-35 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In claim 14, "The protein kinase substrate of claim 1" does

not find antecedent basis in claim 1. In claims 25 and 27, it is not clear if the "particular, randomized amino acid sequence" or "particular, randomized core nucleic acid sequence" can be any sequence or they must all conform to the conditions found in the subparagraph beginning with "a second means for labeling". For examination purposes, the core randomized language is being treated by examiner as not subject to the requirements found in the second means for labeling subparagraph since the language doe not require any specific sequence or limit the sequence to any specific target enzyme. In claim 34 the biomolecular substrate is of a different scope than the protein kinase substrate of claim 14. In claim 35, "said tetramethylrhodamine-5-maleimide" and "SEQ ID NO. 7" do not have antecedent basis in claims 1, 10 or 31.

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

7. Claims 1-2, 5-7, 15 and 21 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Odom. In the paper Odon discusses an apparent conformational change in phenylalanine transfer RNA that is associated with the peptidyl transferase reaction. Fluorescence techniques were used to detect changes in the conformation of $tRNA^{Phe}$ that may occur during the peptidyl transferase reaction in which the tRNA appears to move between binding sites on ribosomes. Such a conformational change may be a fundamental part of the translocation mechanism by which tRNA and mRNA are moved through ribosomes. Escherichia coli $tRNA^{Phe}$ was specifically labeled on acp^3U_{47} and s^4U_8 or at the D positions 16 and 20. The labeled tRNAs were bound to ribosomes as deacylated $tRNA^{Phe}$ or AcPhe-tRNA. Changes in fluorescence quantum yield and anisotropy were measured upon binding to the ribosomes and during the peptidyl transferase reaction. In one set of experiments ***non-radiative energy transfer was measured between a coumarin probe at position 16 or 20 and a fluorescein attached to acp^3U_{47} on the same $tRNA^{Phe}$ molecule***. This constitutes the at least partial quenching due to

non-fluorescence resonance energy transfer. The results indicate that the apparent distance between the probes increases during deacylation of AcPhe-tRNA as a result of peptide bond formation. All of the results are consistent with but in themselves do not conclusively establish that tRNA undergoes a conformational change as well as movement during the peptidyl transferase reaction. See the various tables and results and discussion sections. In particular page 933 discusses how the fluorescence of the coumarin label changes as the environment of the singly labeled substrate changes. Also pages 934-935 discuss the energy transfer and its use in determination of conformational changes in molecules by measuring changes in the distance between the two labels of the substrate. The two labels exemplified are instantly claimed in claims 5-6.

8. Claims 1-2, 5-7, 15 and 21 are rejected under 35 U.S.C. 102(e) as being clearly anticipated by Gilda (US 6,485,901). In the patent Gildea teaches methods, kits and compositions of Linear Beacons containing energy-transferring donors and acceptors and their use in nucleic acid hybridization. Linear Beacons are polymers containing donor and acceptor moieties separated by a nucleobase sequence. The polymers do not necessarily form a stem-loop hairpin. The efficiency of energy transfer between the donor and acceptor moieties is substantially independent of at least two of the following variables: sequence length, spectral overlap of donor and acceptor, presence or absence of Mg, and ionic strength of the solution. Preferred linear beacons are fluorophore-containing peptide nucleic acids (PNAs). In the absence of a target sequence, Linear Beacons facilitate efficient energy transfer between the donor and acceptor moieties linked to opposite ends of the probe. Upon hybridization of the probe to a target sequence, there is a measurable change in at least one property of at least one donor or acceptor moiety of the probe which can be used to detect, identify or quantitate the target sequence in a sample. Experiments demonstrating the non-FRET behavior of the PNA Linear Beacons and their use in detection of Pseudomonas aeruginosa and Bacillus subtilis as well as detection of PCR-amplified K-ras gene were demonstrated. Tables 1B and 1C show Linear Beacons preparation. The structure of column 12 includes several covalently modifiable groups.

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

10. Claims 1-7, 10, 14-33 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Blumenthal in view of Odom as explained above, the admission of prior art as found on page 10, lines 15 to 30 and Tyagi (either (WO 97/39008 or US 6,150,097). In the paper Blumenthal reviews the development and characterization of fluorescently-labeled myosin light chain kinase calmodulin-binding domain peptides. The development and characterization of peptides based on the sequence of the calmodulin-binding domain of skeletal muscle myosin light-chain kinase which were labeled with the fluorescent reagent, acrylodan are described. The use of these fluorescently-labeled peptides to study various aspects of calmodulin-peptide interactions including binding affinity, stoichiometry, specificity, changes in peptide conformation, and thermal stability of the peptide-calmodulin complex is demonstrated. Page 46 discusses the preparation of analogs by replacing different amino acids within a natural peptide sequence to examine these properties. Blumenthal also teaches the formation of a library of peptides with different fluorescent labels. Page 46 also discusses the change in the acrylodan fluorophore as the environment changes and how this is useful in determining various properties. The peptides exhibit many of the salient features seen with calmodulin-target enzyme interactions. The fluorescently-labeled peptides should serve as useful models for studying calmodulin-target enzyme interactions at the molecular level. Blumenthal does not teach the use of a substrate that is doubly labeled with labels that have at least a part of their energy transfer through non-radiative pathways.

On page 10 of the instant specification SEQ ID NOS. 1, 2, 3, 4 and 9 are disclosed as known in the prior art (see the references to patent and literature sources).

Since the Tyagi US Patent is a continuation of the application that resulted in the WO publication, only the US Patent will be discussed by reference the parts of the reference. In the patent Tyagi teaches detection of probes in nucleic acid hybridization using non-fluorescence resonance energy transfer (non- FRET) pairs of chromophores. Nucleic acid hybridization probes are described having a first conformation when not interacting with a target and a second conformation when interacting with a target, and having the ability to bring a label pair into touching contact in one conformation and not the other, are labeled with a non-FRET pair of chromophores and generate a fluorescent or absorbance signal. As opposed to FRET, quenching molecules and even other fluorophores can serve as efficient quenching moieties for fluorophores when attached to nucleic acid hybridization probes such that the fluorescing moiety and quenching moiety are in contact, even when the rules of FRET are violated. To demonstrate probes with "touching" pairs of a fluorophore with another fluorophore or quencher, where the pairs are not FRET pairs, fluorescence quenching efficiency was measured where Molecular Beacon probes were end-labeled with DABCYL at one end and one of 8 different fluorophores at the other end. DABCYL could quench the fluorescence of fluorescein, Lucifer Yellow, BIDIPY, eosine, erythrosine, tetramethylrhodamine, Texas Red, and coumarin. Effective fluorophore quenching also occurred in non-FRET pairs containing other quenchers, DABMI and Malachite Green, as well as appropriate (shorter wavelength) fluorophores such as coumarin. The utility of fluorophore-quencher combinations is demonstrated in a multiplex detection assay using 4 different nucleic acid targets. Columns 1-3 discuss the use of FRET labeled substrates in the prior art and notes that a disadvantage is related to the requirement for overlap between the labels in order to produce the desired affect. Columns 3-4 discuss the advantages of the non-FRET label pairs in that they do not require the overlap of FRET pairs and can therefore provide measurable results or enhancement even when FRET results are not possible.

It would have been obvious to one of skill in the art at the time of the invention to incorporate a double label selected from those taught by Odom or Tyagi in the Blumenthal substrates or with a substrate known in the prior art to have a structure modifiable by the enzymes as admitted in the instant specification for their recognized ability to determine

conformational changes and work in situation that FRET label pairs or single labels are not able to provide that information as taught by Odom and Tyagi.

11. Claims 1-7, 10, 14-33 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Macala, McIlroy, Schultz (US 5,580,747) or Ventura in view of Blumenthal or Wei (analytical chemistry 1994) and Odom, the admitted prior art or Tyagi as explained above.

In the paper Macala teaches measurement of cAMP-dependent protein kinase activity using a fluorescent-labeled Kemptide. Traditional protein kinase assays include the use of ^{32}P-labeled ATP as phosphate donor and a substrate protein or peptide as phosphoreceptor. Since this approach has a number of drawbacks in addition to generating ionizing radiation, several non-isotopic methods have been developed. Although shown to reflect the activity of purified enzymes, none have been demonstrated to detect physiological changes in endogenous enzyme activity in cell homogenates. Studies were performed to examine the kinetics, reproducibility, and optimal assay conditions of a novel non-radioisotopic kinase assay that detects protein kinase A (PKA) activity by phosphorylation of the peptide substrate, Kemptide, covalently bound to a fluorescent molecule (fluorescamine-labeled Kemptide; f-Kemptide). Fluorescence was determined by spectrofluorometry with excitation at 568 nm and emission at 592 nm. Basal and agonist-induced PKA activities in epithelial cell homogenates were measured. The kinetics of f-Kemptide were similar to the standard radioisotopic method with intra-assay and inter-assay variations of 5.6 ± 0.8% and 14.3 ± 2.6%, respectively. Neither fluorescence quenching nor enhancing effects were found with consistent amounts of homogenate protein. Specific PKA activity was determined as the IP20-inhibitable fraction to account for nonspecific phosphorylation, perhaps due to S6 kinase or a similar enzyme. The basal activity of 38% of total PKA in A6 cells increased by 84% after exposure to vasopressin and by 58% after short exposure to forskolin. In T84 cells exposed to VIP there was a 360% increase over basal activity. These results show that f-Kemptide exhibits acceptable kinetics, and that the assay system can quantitatively and reproducibly measure basal and stimulated PKA activity in cell homogenates. Macala does not teach the substrate having two dyes attached or a library of compounds.

In the paper McIlroy teaches a continuous fluorescence assay for protein kinase C. A 6-acryloyl-2-dimethylaminoapthalene (acrylodan)-labeled 25-amino acid peptide (acrylodan-

CKKKKRFSFKKSFKLSGFSFKKNKK-COO-), containing the protein kinase C (PKC) phosphorylation sites of brain myristoylated alanine-rich kinase C substrate (MARCK) protein, undergoes a 20% fluorescence decrease when it is phosphorylated by PKC. This fluorescence decrease is dependent on the presence of PKC, Ca^{2+} (half-maximal stimulation at pCa = 6.2), phosphatidylserine, diacylglycerol, or phorbol-12-myristate-13-acetate (half-maximal stimulation at 2 nM) and ATP, and correlates well (r = 0.007) with [^{32}P]phosphate incorporation into the peptide. This fluorescence assay allows detection of 0.02 nM PKC, whereas similar concentrations of cAMP-dependent or type II calmodulin-dependent protein kinases produced no change in peptide fluorescence. The method can be used to assay purified PKC as well as activity in crude brain homogenates. Incubation of PKC with staurosporine inhibits the fluorescence decrease with an IC50 of 2 nM. Thus, the fluorescence decrease that occurs in the acrylodan-peptide provides a continuous fluorescence assay for PKC activity. The assay is taught as an alternative, non-radioactive, continuous (homogeneous) assay for PKC (page 148 in paragraph bridging the columns). McIlroy does not teach a doubly-labeled substrate.

In the patent Shultz teaches a non-radioactive assay and purification of proteins, and particularly to the non-radioactive assay and purification of protein kinases, phosphatases and protease by incubating the enzyme with a substrate modified peptide to form a product modified peptide under conditions where the enzyme is active. The product modified peptide and substrate modified peptide are then separated, and the product modified peptide is measured. The present invention is also directed to kits and bioreagents for performing the assays. Table 1 of the patent shows a list of substrates that have a fluorescent dye attached to the substrate. Shultz does not teach the substrate having two dyes attached or a library of compounds.

In the paper Ventura teaches phorbol ester regulation of opioid peptide gene expression in myocardial cells and the role of nuclear protein kinase C. Opioid peptide gene expression was characterized in adult rat ventricular cardiac myocytes that had been cultured in the absence or the presence of phorbol 12-myristate 13-acetate. The phorbol ester induced a concentration- and time-dependent increase of prodynorphin mRNA, the maximal effect being reached after 4 hours of treatment. The increase in mRNA expression was suppressed by incubation of cardiomyocytes with staurosporine, a putative protein kinase C inhibitor, and was not observed when the cells were cultured in the presence of the inactive phorbol ester 4a-phorbol 12,13-

didecanoate. Incubation of cardiac myocytes with phorbol 12-myristate 13-acetate also elicited a specific and staurosporine-sensitive increase in immunoreactive dynorphin B, a biologically active end product of the precursor, both in the myocardial cells and in the culture medium. In vitro run-off transcription assays indicated that transcription of the prodynorphin gene was increased both in nuclei isolated from phorbol ester-treated myocytes and in nuclei isolated from control cells and then exposed to phorbol 12-myristate 13-acetate. No transcriptional effect was observed when cardiac myocytes or isolated nuclei where exposed to 4a-phorbol 12,13-didecanoate. The phorbol ester-induced increase in prodynorphin gene transcription was prevented by pretreatment of myocytes or isolated nuclei with staurosporine, suggesting that myocardial opioid gene expression may be regulated by nuclear protein kinase C. In this regard, cardiac myocytes expressed protein kinase C-α, -δ, -ε, and -ζ, as shown by immunoblotting. Only protein kinase C-δ and protein kinase C-ε were expressed in nuclei that have been isolated from control myocytes, suggesting that these 2 isotypes of the enzyme may be part of the signal transduction pathway involved in the effect elicited by the phorbol ester an opioid gene transcription in isolated nuclei. The incubation of myocardial nuclei isolated from control cells in the presence of a protein kinase C activator induced the phosphorylation of the myristylated alanine-rich protein kinase C substrate peptide, a specific fluorescent substrate of the enzyme. The possibility that prodynorphin gene expression may control the heart function through autocrine or paracrine mechanisms is discussed. Ventura does not teach the substrate having two dyes attached or a library of compounds.

In the paper Blumenthal reviews the development and characterization of fluorescently-labeled myosin light chain kinase calmodulin-binding domain peptides. The development and characterization of peptides based on the sequence of the calmodulin-binding domain of skeletal muscle myosin light-chain kinase which were labeled with the fluorescent reagent, acrylodan are described. The use of these fluorescently-labeled peptides to study various aspects of calmodulin-peptide interactions including binding affinity, stoichiometry, specificity, changes in peptide conformation, and thermal stability of the peptide-calmodulin complex is demonstrated. Page 46 discusses the preparation of analogs by replacing different amino acids within a natural peptide sequence to examine these properties. Blumenthal also teaches the formation of a library

of peptides with different fluorescent labels. Page 46 also discusses the change in the acrylodan fluorophore as the environment changes and how this is useful in determining various properties. The peptides exhibit many of the salient features seen with calmodulin-target enzyme interactions. The fluorescently-labeled peptides should serve as useful models for studying calmodulin-target enzyme interactions at the molecular level.

In the paper Wei teaches antibody-mediated fluorescence enhancement based on shifting the intramolecular dimer/monomer equilibrium of fluorescent dyes. A novel concept is described for directly coupling fluorescence emission to protein-ligand binding. It is based on shifting the intramolecular monomer/dimer equilibrium of two fluorescent dyes linked by a short spacer. A 13-residue peptide, recognized by a monoclonal antibody against human chorionic gonadotropin (hCG), was labeled with fluorescein (F) and tetramethylrhodamine (T) at its N- and C-termini, respectively. Spectral evidence suggests that when the conjugate is free in solution, F and T exist as an intramolecular dimer. Fluorescence quenching of fluorescein and rhodamine is ~98% and ~90%, respectively, due to dimerization. When the double-labeled peptide is bound to anti-hCG, however, the rhodamine fluorescence increases by ≤7.8-fold, depending upon the excitation wavelength. This is attributed to the dissociation of intramolecular dimers brought about by conformational changes of the conjugate upon binding. Fluorescein fluorescence was still quenched because of excited-state energy transfer and residual ground-state interactions. Antibody binding also resulted in a ~3.4-fold increase in fluorescence anisotropy of the peptide. These changes in intensity and anisotropy allow direct measurement of antigen-antibody binding with a fluorescence plate reader or a polarization analyzer, without the need for separation steps and labeling antibodies. Because recent advances in peptide technology have allowed rapid and economical identification of antigen-mimicking peptides, the double-labeled peptide approach offers many opportunities for developing new diagnostic assays and screening new therapeutic drugs. It also has many potential applications to techniques involving recombinant antibodies, biosensors, cell sorting, and DNA probes. The second full paragraph of page 1501 teaches that the intramolecular monomer/dimer equilibrium is essentially a molecular switch that can be toggled directly by binding events- process that would otherwise be difficult to achieve with high molecular weight protein antigens. Applications of this technology may well extend beyond clinical immunoassays to other biotechnological areas such as biosensors, DNA probes, enzyme

assays, recombinant antibodies, peptide libraries, and 2D molecular assembly. Relative to the instant claims is the listing of enzyme assays and the first full paragraphs of pages 1501 and 1503 explaining how the dimers are produced.

It would have been obvious to one of skill in the art at the time of the invention to incorporate a double label selected from those taught by Odom, Tyagi or Wei in the Macala, Shultz or Ventura substrates because of the ability to detect conformational changes in the substrate due to covalent modifications of the substrates as shown by Odom and Tyagi or the lack of a need to perform a separation step as taught by Wei. One of skill in the art would also have recognized that libraries of substrates as taught by Blumenthal or Wei or the admitted known structures of the prior art would have allowed the Macala, Shultz or Ventura substrates to be used for characterizing enzyme properties as shown by Blumenthal and used in enzyme assays which are taught by Wei as an extension of the concepts used in the immunoassays.

12. Applicant's arguments filed April 30, 2007 have been fully considered but they are not persuasive. Relative to the lack of enablement, examiner points out that the expectation of success is a relative thing irrespective of whether applicant used means-plus-function language. Examiner is not saying that the invention is not enabled, however the invention is not enabled for the scope that applicant is trying to claim. Whether applicant chooses to use means-plus-function or the type of claim language used in the prior response, the requirement is that the scope claimed must be enabled. In the instant case, the expectation of successfully developing other workable compounds besides the one compound described in example 1, is dependent on a number of things. These include the fact that the dye combinations can be involved in molecules that result in either or both FRET and non-FRET quenching. To meet the claim limitations, it requires at least four additional things to be present: a molecular backbone, a group or position in the backbone capable of being covalently modified that causes a change in the conformation of the molecule, a fluorescent dye and a second dye that are both covalently bonded to the molecular backbone in a configuration that the second dye quenches the fluorescence of the fluorescent dye through a ground state interaction before covalent modification of the molecule and allows fluorescence after covalent modification of the molecule. Variables in this system include the form or structure of the backbone, the positioning of the two dyes along the backbone and the dye combinations used. The backbone must have two things a structure that has at least

two possible conformations and a portion of the backbone that is modifiable without cleavage of the backbone to control which conformation is present. The instant claims do not limit the form of the backbone and include biological types of structures as well as non-biological structures. The next level of difficulty is added with the placement of two dyes along the backbone at positions that they will interact (include quenching) in one configuration of the backbone and not interact in the second configuration of the backbone. The third level of difficulty comes in that not only must the dyes interact, but they must interact with at least some degree of non-FRET quenching present. If every dye combination has the presence of non-FRET quenching, then this is not a difficult problem. It is not this basis that the art rejections are based. If the presence of non-FRET quenching requires that the dyes have a certain or specific structural relationship for non-FRET quenching to occur, then not every position along a molecular backbone will be capable of producing the proper conditions or configuration for the non-FRET quenching to occur. Focusing more on the embodiments shown, there are a number of sequences that are known to be susceptible to modification by a phosphorylating enzyme. However, how many of these sequences would be expected by one of ordinary skill in the art to be modifiable to include the two dyes needed and include a change in conformation that is capable of causing the two dyes to have an interaction that includes non-FRET quenching in one of the conformations and the absence of that quenching in the second conformation. If a non-FRET quenching requires the dyes to have a specific structural relationship, how easy is it to predict which of the many possible sites to attach the two dyes will lead to a configuration that will produce the non-FRET quenching rather than the FRET quenching which always appears to be a possibility. In other words it appears that FRET quenching is more likely that non-FRET quenching. However as shown above with the Mizukami reference, molecules designed for FRET-quenching can have non-FRET quenching and the predictability is low. This clearly decreases the expectation that non-FRET quenching will be present. It appears that a large number of potential sequences will lead to only a few compounds that meet the requirements of the claims. Additionally it appears that the effort to find compounds that meet the claim requirements is great with little expectation of success. Thus it appears that even in spite of the few examples shown in the specification, the discovery of additional molecules that work requires a large amount or trial an error work. Thus the specification is not enabled for the scope that is claimed. Additionally, applicant has not

shown that their own work has produced compounds beyond that exemplified in the specification or that others have, as a benefit of applicant's teachings, developed compounds within the instant claim scope that are not exemplified in the instant specification.

The cited Schobel reference shows that either one of two things are possible. The first is that even in situation in which the donor-acceptor pair of dyes are designed for efficient FRET quenching (nearly total spectral overlap), there are ground state interactions (static effects) that lead to a part of the quenching that occurs. This would lead one to an expectation of an inherent presence of a ground state interaction that is responsible for at least a part of the quenching that occurs. Thus there is at least an expectation that there is ground state interactions involved in the quenching of Odom. Additionally, the dyes of Odom are clearly found in the listing of dyes found in claims 5-6. This also would have led one in the art to an expectation of the presence of a ground state interaction that is involved in at least a part of the quenching. The second possibility is that the presence of ground state interactions that lead to quenching are not predictable. Particularly when coupled with a covalent modification of the molecule that is required to change the conformation of the molecule and as a result change the amount of quenching that occurs. Relative to the first possibility, if applicant's invention is the discovery of the ground state interactions involved in the a known quenching process, then the claims should be limited to molecules that are not known or obvious based on that which is known. Relative to the second possibility, due to the unpredictability of the presence of ground state interactions and the limited number of examples in the instant specification, the claims should be limited to those that are exemplified as actually working in the instant specification.

Relative to the combination of references used in the obviousness rejections, examiner agrees that those references not used as anticipatory references do not anticipate the claims. Examiner points out that there is no limitation placed on the form that the backbone may take and a natural or synthetic polypeptide, protein or polynucleic acid is within the claim scope as is a polymer or other type of oligomer. Additionally, examiner notes that there is a difference in the behavior when the molecule is associated with another molecule such as shown in Odom. Examiner also points out that even though applicant may have recognized another advantage which would flow naturally from following the suggestion of the prior art, this cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*,

227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985). In other words, if the modification suggested as being obvious by a combination of references has additional reasons for making the modification that applicant has discovered, the obviousness of the combination does not change. The above discussion regarding the presence of ground state interactions that causes quenching in molecules designed for FRET quenching is also relevant to the obviousness of the claims. Additionally the Tyagi reference clearly shows advantages of using non-FRET label pairs. Thus there is sufficient motivation and/or teachings in the applied references to either anticipate or obviate the claims. The applied McIlroy and Wei references add to the combinations in that they clearly show that fluorescence is measurable due to the action of an enzyme on its substrate and that conformational changes as are found in enzymes can be the basis for a molecular switch where the conformational changes affect a dimer/monomer equilibrium for two fluorescent dyes bonded to a substrate.

Relative to the Odom and Gildea references, examiner notes two things: first, the claims only require that the fluorescence is partially quenched through a non-FRET mechanism and second, that a particular compound can have properties and/or capabilities that are not taught or recognized by the prior art in which they are described. Since the fluorescence of the claimed molecules is required to be only partially quenched by a non-FRET mechanism, the fluorescence could be mostly quenched by another mechanism such as FRET. In other words any quenching that is by a non-FRET mechanism is within the scope of partially quenched through a non-FRET mechanism. Because of this, an argument that the reference does not teach that the quenching mechanism is non-FRET is not persuasive. As noted above the cited Schobel reference shows that it is possible for a donor-acceptor pair of dyes that are designed for efficient FRET quenching (nearly total spectral overlap), to have ground state interactions (static effects) that lead to a part of the quenching that occurs. Also the above noted Mizukami reference shows that a dilabeled compound that was designed for FRET quenching can have a dimer configuration that has mostly non-FRET quenching. As a result, applicant is required to show or explain why the quenching mechanism does not include any quenching by a non-FRET mechanism.

Relative to the combination of references, examiner notes that applicant is not arguing that the peptide substrates of Blumenthal do not contain a site that is covalently modifiable, that a dilabeled complex using the teachings of Odom or Tyagi is not within the claimed scope or that

there is not motivation for adding a second label to the compound(s) taught by Blumenthal. Therefore examiner is understanding this to mean that there is sufficient motivation in the reference combination to add a second dye to the compound(s) of Blumenthal. In response to applicant's argument that the references do not teach that the compound(s) have non-FRET quenching or have non-FRET quenching only due to a feature that is found in polynucleic acids, the fact that applicant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*, 227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985).

13. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additionally cited art relates to static or ground state fluorescence quenching and enzyme modulated conformational changes in a substrate.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose telephone number is (571) 272-1265. The examiner's schedule is variable between the hours of about 6:30 AM to about 5:00 PM on Monday through Thursday and alternate Fridays.

A general phone number for the organization to which this application is assigned is (571) 272-1700. The fax phone number to file official papers for this application or proceeding is (571) 273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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